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Defibrotide for the Treatment of Severe Hepatic Veno-Occlusive Disease and Multiorgan Failure after Stem Cell Transplantation: A Multicenter, Randomized, Dose-Finding Trial

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Therapeutic options for severe hepatic veno-occlusive disease (VOD) are limited and outcomes are dismal, but early phase I/II studies have suggested promising activity and acceptable toxicity using the novel polydisperse oligonucleotide defibrotide. This randomized phase II dose-finding trial determined the efficacy of defibrotide in patients with severe VOD following hematopoietic stem cell transplantation (HSCT) and identified an appropriate dose for future trials. Adult and pediatric patients received either lower-dose (arm A: 25 mg/kg/day; n = 75) or higher-dose (arm B: 40 mg/kg/day; n = 74) i.v. defibrotide administered in divided doses every 6 hours for ≥14 days or until complete response, VOD progression, or any unacceptable toxicity occurred. Overall complete response and day +100 post-HSCT survival rates were 46% and 42%, respectively, with no significant difference between treatment arms. The incidence of treatment-related adverse events was low (8% overall; 7% in arm A, 10% in arm B); there was no significant difference in the overall rate of adverse events between treatment arms. Early stabilization or decreased bilirubin was associated with better response and day +100 survival, and decreased plasminogen activator inhibitor type 1 (PAI-1) during treatment was associated with better outcome; changes were similar in both treatment arms. Defibrotide 25 or 40 mg/kg/day also appears effective in treating severe VOD following HSCT. In the absence of any differences in activity, toxicity or changes in PAI-1 level, defibrotide 25 mg/kg/day was selected for ongoing phase III trials in VOD.

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INTRODUCTION

Hepatic veno-occlusive disease (VOD) is one of the more common and important nonhematologic toxicities following high-dose cytoreductive therapy for hematopoietic stem cell transplantation (HSCT) [1,2]. Diagnosis of hepatic VOD is based on clinical criteria, including the presence of painful hepatomegaly,

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jaundice, ascites, fluid retention, and weight gain [2,3]. The condition usually develops before day +30 post-HSCT, although it can occur later [1,2,4]; its reported incidence varies from ~5% to 60%, and it is typically associated with the conditioning regimen used as well as the donor source, being more common after allogeneic HSCT than after autologous HSCT [4-7]. The presentation of VOD ranges from mild, reversible disease to a severe syndrome associated with multiorgan failure (MOF) and death [4-6]. Severe VOD is one of the most frequent causes of early death in the HSCT setting, with a mortality rate of up to 98%–100% by day +100 post-HSCT in adults, and marginally lower mortality in children [5,6,8].

Hepatic VOD is also known as sinusoidal obstruction syndrome (SOS) or VOD/SOS, reflecting the primary injury typically caused by cytoreductive agents to sinusoidal endothelial cells in zone 3 of the hepatic acinus [9,10]. Secondary events include sinusoidal hemostasis leading to elevated sinusoidal pressure and dilatation, subendothelial edema within small hepatic venules, and deposition of fibrinogen and factor VIII in both sinusoids and within hepatic venules [9,11]. These events are followed by ischemia, hepatocyte necrosis, collagen deposition, and sinusoidal fibrosis, leading to sinusoidal obstruction followed by sclerosis and occlusion of hepatic venules [9-11]. As these processes progress, widespread intrahepatic zonal disruption leads to portal hypertension, worsening liver dysfunction, and ascites, eventually resulting in MOF (characterized by pulmonary and renal dysfunction, as well as encephalopathy) and death [10,11].

Current standard treatments for hepatic VOD are supportive, including diuresis, transfusion, renal replacement therapy, and analgesia. There are few effective options that target the underlying cause. Reduced-intensity conditioning regimens, individualized chemotherapy dosing, and avoidance of cyclophosphamide (Cy) are currently experimental approaches to prevention, but these strategies often are not suitable for chemoresistant hematologic malignancies [1]. Administration of intravenous busulfan and pharmacokinetic (PK) monitoring of busulfan and Cy have been shown to reduce the frequency of VOD [12]. Prophylactic ursodeoxycholic acid use has been associated with decreased incidence of VOD in some studies, although the relative effects on cholestatic versus sinusoidal injury per se are unclear [13]. Currently, there is no proven effective therapy for either prevention or treatment of hepatic VOD. Interventional studies that have investigated prostaglandin E1, tissue-plasminogen activator (t-PA), heparin, and antithrombin III (ATIII) for treating severe VOD have demonstrated neither significant effectiveness nor safety in this setting [14-16]. Indeed, the use of t-PA in patients with severe VOD and MOF has been associated with markedly excessive toxicity, precluding its use in this population [14].

Defibrotide is a polydisperse oligonucleotide with local antithrombotic, anti-ischemic, and anti-inflammatory activity. It binds to the vascular endothelium, modulates platelet activity, promotes fibrinolysis, decreases thrombin generation and activity, and reduces circulating levels of plasminogen activator inhibitor type 1 (PAI-1) [17-21]. Defibrotide has selective and protective effects on the small vessels, but not macrovascular, endothelium [22,23]. Experimental models suggest that it may enhance endothelial cell survival and stabilize microvasculature [24]. Defibrotide has no protective effect on tumors, and in fact was found to enhance the antitumor activity of various agents in preclinical studies [25]. In adult and pediatric patients with VOD, defibrotide treatment has been associated with complete response (CR) rates of 36%–76%, with day +100 post-HSCT survival rates of 32%–79% and no substantial defibrotide-associated toxicity [26-30]. Multicenter phase I/II trials of defibrotide have included intrapatient dose escalation from 10 mg/kg/day to 60 mg/kg/day [26-28]. In the largest study published to date, the majority of CRs occurred at doses of 20-40 mg/kg/day [28].

The objectives of our dose-finding trial were to (1) determine the CR rate in patients with severe VOD following HSCT treated with defibrotide at 25 or 40 mg/kg/day, (2) assess the safety profile of defibrotide at these doses in this population, and (3) determine the dose for use in phase III and other future trials in VOD. The trial also aimed to generate preliminary descriptive PK data for defibrotide in a limited subset of patients.

MATERIALS AND METHODS

Patients

Adult or pediatric patients with a clinical diagnosis of hepatic VOD, defined as the presence of jaundice (total serum bilirubin ≥2 mg/dL) and at least 2 associated signs, including ascites, weight gain >5% from baseline, hepatomegaly, or right upper quadrant pain, by day +35 post-HSCT were considered eligible. Abdominal Doppler ultrasound was performed at trial entry to identify the presence/absence of portal vein blood flow reversal and confirm diagnostic findings. Patients with jaundice and portal vein blood flow reversal on Doppler examination and only one other diagnostic criteria were eligible for the trial. For patients with preexisting hepatomegaly, confirmation of liver size increase after admission by physical examination or imaging was required. Patients who did not meet all criteria but had biopsy-proven VOD were also eligible. In patients with concurrent confounding causes of liver dysfunction, positive biopsy findings or a wedged transhepatic venous pressure gradient ≥10 mm Hg was required to confirm

eligibility, with best medical judgment and further imaging studies used to clarify the diagnosis. All central pathology reviews were performed by a single investigator (H.M.S.). Recuts of all liver specimens from needle biopsies and autopsies were stained at the Seattle Cancer Care Alliance pathology laboratory with hematoxylin and eosin, Mason trichrome, and Gordon-Sweet reticulin stains. Hepatic VOD was diagnosed based on the finding of hepatic venules with luminal narrowing from subendothelial edema and entrapped red cells or connective tissue. A small specimen without adequate hepatic venules was not considered evaluable even if it exhibited other stigmata of VOD, such as sinusoidal fibrosis and centrilobular hemorrhagic necrosis.

Patient eligibility was also determined according to the Bearman criteria and/or the presence of MOF. Patients assessed based on the Bearman model [31] had to have a predicted ≥30% risk of severe VOD. As described previously [28], patients not assessed by the Bearman model were eligible if they had concomitant MOF (presence of renal, pulmonary, and/or central nervous system dysfunction). MOF was defined as follows:

- Renal: creatinine ≥2 times the level at admission for conditioning or ≥2 times the lowest level during conditioning, or creatinine clearance or glomerular filtration rate ≤50% the level at admission, or dialysis dependence
- Pulmonary: oxygenation saturation ≤90% on room air and/or the need for positive pressure/ventilator dependence not attributable to any other cause
- Central nervous system: confusion, lethargy, and/or delirium not attributable to any other cause.

Exclusion criteria included significant uncontrolled acute bleeding; hemodynamic instability requiring multiple vasopressors; grade B-D graft-versus-host disease (GVHD) according to the International Bone Marrow Transplant Registry Severity Index [32], excluding grade B skin-only GVHD; intubation for documented intrinsic lung disease (eg, focal pneumonia), excluding intubation secondary to a mechanical barrier to ventilation in the presence of adequate oxygenation parameters (partial pressure of oxygen [PaO_2]/fraction of inspired oxygen [FiO_2] ratio ≥300 and/or oxygen index [OI = (mean arterial pressure × FiO_2)/PaO_2 × 100] ≤25%) at enrollment; grade 4 (National Cancer Institute's CTC version 2.0) neurotoxicity, excluding confusion and/or delirium; previous or concomitant systemic t-PA therapy; concomitant use of heparin or other anticoagulants (discontinuation of heparin treatment ≥12 hours before enrollment rendered patients eligible), excluding use for routine central venous line (CVL) management, fibrinolytic instillation for CVL occlusion, intermittent dialysis, or ultrafiltration; and current treatment with another experimental agent. Concomitant treatment with antithrombin III or other antithrombotics, nonsteroidal anti-inflammatory drugs, or ursodiol was not permitted (although ursodiol could be used in specific circumstances, such as radiologically confirmed bile sludging).

Study Design

This was a multicenter, randomized, dose-finding, opened-label, phase II trial performed at 6 U.S. sites between April 2000 and April 2006 (NCT00003966; ClinicalTrials.gov). Patients were randomized to lower-dose (arm A) or higher-dose (arm B) defibrotide (Gentium, Como, Italy). Randomization was stratified by whether or not the conditioning regimen included Cy and by age (adult [≥18 years] vs pediatric [<18 years]). For both treatment arms, the defibrotide starting dose was 2.5 mg/kg every 6 hours for 4 doses (total dose, 10 mg/kg), based on baseline weight, defined as weight on the date of admission to the transplantation unit for conditioning. This initial dose was intended to ensure tolerance before dose escalation. The dose was then increased to 6.25 mg/kg every 6 hours (total dose, 25 mg/kg/day) in arm A and to 10 mg/kg every 6 hours (total dose 40 mg/kg/day) in arm B. Defibrotide was administered i.v. in 5% dextrose in water, mixed to a maximum concentration of 4 mg/mL, with the dose rounded to the nearest 10 mg. Patients were treated for a minimum of 14 days or until achievement of CR, or until progression of VOD, unacceptable toxicity (recurrent grade 3/4 adverse events [AEs] considered likely or definitely related to defibrotide), or comorbidities precluded further treatment. During therapy, wherever possible, transfusions were used to maintain platelets ≥20,000/μL and hematocrit ≥30% (although transfusion support was not formally monitored as part of the study), with factor replacement targeting a prothrombin time ≤15 seconds and fibrinogen ≥150 mg/mL. Other appropriate supportive care was continued as necessary, although routine use of low-dose dopamine was discouraged because it could increase splanchnic pooling, reducing renal plasma flow. The study protocol was approved by an independent institutional review board at each participating site. All trial participants (or their parents/guardians/health care proxy) gave written informed consent.

Assessments

The primary endpoint was CR rate. CR was defined as total serum bilirubin <2 mg/dL after initiation of defibrotide with resolution of VOD-related MOF. Resolution of renal, pulmonary, and central nervous system dysfunction were defined as, respectively, creatinine decrease to <2 times baseline and/or resolution of dialysis dependence, resolution of oxygen requirement, and resolution of encephalopathy.

Secondary endpoints included safety and tolerability of defibrotide, effect of defibrotide on PAI-1, and

relationship between defibrotide dose and response, day +100 mortality, and/or PAI-1 level. Patients were followed for evidence of expected AEs (including bleeding; allergic reactions; vasomotor effects including flushing, dizziness, headache, and hypotension; nausea; vomiting; diarrhea; and fever) and defibrotide-attributable grade 3/4 end-organ dysfunction. Blood samples for analysis of PAI-1 and bilirubin levels were obtained at baseline, twice weekly during therapy, and at the end of treatment.

Additional planned objectives were to determine the feasibility of PK analysis and to generate some preliminary descriptive PK data for defibrotide in a subset of patients. These study objectives were added by amending the protocol after almost half of the patients had been enrolled, and thus they were an optional part of the protocol, limited to a small number of patients. Blood samples for PK analysis were obtained on treatment days 1, 2, and 7 (for adults: 12 samples/day, just before the start of the first infusion; at 30, 60, 90, and 115 minutes after the start of first infusion; and at 5, 10, 15, 20, 30, 45, and 60 minutes after the end of the first infusion; for pediatric patients: 2 samples/day, just before the start of the first infusion and 115 minutes after the start of the first infusion). PK samples were analyzed centrally using high-performance liquid chromatography and agarose gel methods.

Abdominal Doppler ultrasound was performed at study entry, on day 14, and after the completion of therapy. Ultrasound images were reviewed locally and then centrally by a single investigator (D.N.D.S). The liver was assessed for size, with the right, left, and caudate lobes measured separately. The gallbladder was evaluated for sludge and wall thickness. Ascites were graded as absent, trace/small, or moderate/large. Portal vein Doppler ultrasonography was performed on the main, right, and left portal veins; pulsed Doppler tracings of each segment were graded in terms of the presence/absence of pulsatility and flow direction. Hepatic artery resistive indices, as well as hepatic vein tracings, were obtained. Pathology assessments were performed locally on available liver tissue and reviewed centrally as detailed previously.

Statistical Analyses

The planned sample size was 140 patients (70 patients/arm). It was anticipated that both arms would exhibit defibrotide activity as assessed by CR rate. The treatment was to be considered promising if the lower limit of the 95% confidence interval (CI) for the CR rate was >18%. With this design, the probability that the treatment would be considered further was 0.82 when the true CR rate was 33%. If the number of observed CRs were 21 of the 70 patients (ie, 30% CR rate) in each arm, then the corresponding 95% CI would be 18%–48% with 70 patients. If, as anticipated, both arms exhibited defibrotide activity, then the less-toxic dose (ie, with a lower rate of severe or worse toxicity) would be taken forward for further investigation. In the absence of any significant differences in activity, toxicity, or change in PAI-1 level, the lower dose would be taken forward.

All randomized patients who received one or more doses of defibrotide (treated population) were included in baseline, survival, and safety analyses. All randomized patients who received 3 or more days of defibrotide therapy (evaluable population) were included in response analyses; this was to allow for administration of 4 initial doses, after which the dose was escalated to 25 or 40 mg/kg/day. It was felt necessary to give patients 1 day (ie, 4 doses) at the initial dose to assess for safety, and at least 1 day (ie, 4 doses) at the treatment dose before evaluating for response, to allow meaningful comparison between arms.

For the primary efficacy analysis, 95% CIs for CR rates by treatment arm were calculated to confirm that the lower limits were >18%. Point estimates with 95% CIs were reported for CR and day +100 post-HSCT survival rates, as well as for the differences in rates between arms. Rates were analyzed in subgroups defined by age (adult/pediatric) and previous therapy. Rates were compared between arms and subgroups using Fisher's exact test to provide descriptive *P* values as a reference. The distributions of time from HSCT or randomization to death were estimated using the Kaplan-Meier method; the log-rank test was applied for comparisons of survival between arms.

PAI-1 and total bilirubin levels were summarized at baseline, day 7, and day 14 of treatment; changes from baseline to days 7 and 14 are reported. The association of radiology and pathology review findings with response and survival was examined as well. Summary statistics are reported for PK data.

RESULTS

Patient Demographics and Disposition

A total of 151 patients were randomized (Figure 1), of whom 149 received one or more doses of defibrotide (arm A: n = 75; arm B: n = 74) and 141 were evaluable for response (arm A: n = 72; arm B: n = 69). Patient demographic and baseline characteristics were well balanced between the 2 arms (Table 1). The majority of patients were caucasian (81% in arm A and 77% in arm B). The characteristics of VOD did nor differ between the 2 arms (Table 2).

Defibrotide Administration

The majority of patients (n = 119; 80%) received their first defibrotide dose on the day of randomization. The median duration of therapy was 19 days (range,

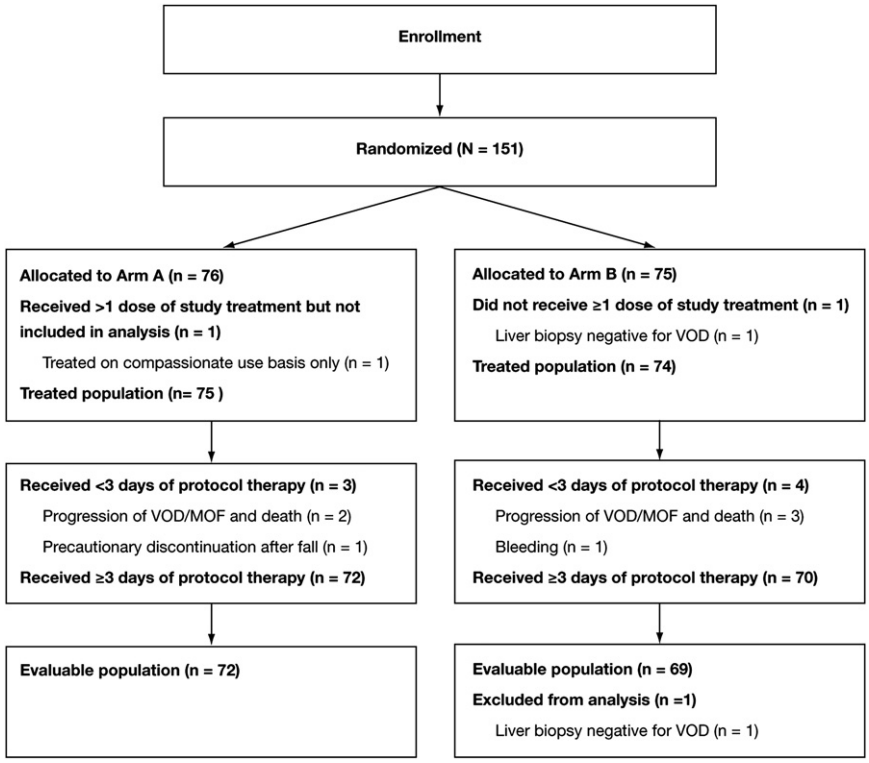

Figure 1. Disposition of patients. Flowchart illustrating the progression of patients with severe hepatic VOD after HSCT by treatment group (arm A, 25 mg/kg/day i.v. defibrotide; arm B, 40 mg/kg/day i.v. defibrotide).

2-82 days) in arm A and 20 days (range, 2-65 days) in arm B. Reasons for ending treatment were resolution of VOD per protocol-defined criteria (33% in arm A and 30% in arm B), VOD progression (15% and 19%), death (13% and 16%), patient discharge (12% and 11%), withdrawal of care (13% and 9%), alternative diagnosis (5% and 3%), and AEs (8% and 12%, of which 3% and 5%, respectively, were considered defibrotide-related toxicity).

Efficacy

The overall CR rate in the evaluable population was 46%, with 49% (95% CI, 37%–69%) in arm A and 43% (95% CI, 32%−55.2%) in arm B (Table 3). The difference between arms (arm B - arm A) of −5% (95% CI: −22% to 11%) was not statistically significant (P = .613). Overall, 42% of the patients in the treated population were alive at day +100 post-HSCT, 44% (95% CI, 33%−55%) in arm A and 39% (95% CI, 28%−50%) in arm B (Table 3). The difference between arms (B - A) of −5% (95% CI, −21% to 11%) was not statistically significant (P = .619). In the patients who died by day +100 post-HSCT, the most common cause of death was progressive VOD (29% in arm A and 28% in arm B). Kaplan-Meier estimates for survival distribution post-HSCT or after randomization demonstrated no notable differences between the 2 arms (Figure 2). In subgroup analyses by age group, the day +100 survival rate in pediatric patients appeared to be higher in arm A (70% vs 36% in arm B; P = .020). Survival distributions by arm and age group showed no significant differences between the 2 arms, although the curves appear to be better for arm A in pediatric patients (Figure 3C and D), consistent with day +100 survival rate data.

In exploratory subgroup analyses, overall CR rate was higher in pediatric patients compared with adult patients (57% vs 40%; P = .056), primarily in arm A (70% vs 39%; P = .015). Overall day +100 post-HSCT survival rate also was higher in pediatric patients (52% vs 37%; P = .074), because of differences in arm A (70% vs 33%; P = .003). The CR rate did not appear to be affected by sirolimus/tacrolimus therapy, but previous exposure to gemtuzumab ozogamicin was associated with a possible trend toward a higher CR rate (58% vs 44%; P = .268). The day +100 survival rate was lower overall (33% vs 47%; P = .092) and also in arm A (29% vs 55%; P = .028) for patients who received sirolimus/tacrolimus; in contrast, previous exposure to gemtuzumab ozogamicin did not appear to be associated with reduced survival (Table 3).

CR and day +100 survival rates were compared by patient MOF status. Assuming the overall CR (46%) and day +100 survival (42%) rates as fixed values, patients who were dialysis- or ventilator-dependent at baseline did not have statistically significantly different CR and day +100 survival rates, whereas those

Table 1. Demographic and Baseline Characteristics (Treated Population) of All Patients and by Treatment Arm

Characteristic	Arm A (n = 75)	Arm B (n = 74)	All Patients (n = 149)
Age group, n (%)			
<18 years (pediatric)	23 (31)	25 (34)	48 (32)
≥18 years (adult)	52 (69)	49 (66)	101 (68)
Median age (range), years	32 (0-61)	34 (0-63)	34 (0-63)
Sex, n (%)			
Male	41 (55)	44 (59)	85 (57)
Female	34 (45)	30 (41)	64 (43)
Weight, kg, median (range)	65.9 (7-126)	65.6 (4-111)	65.8 (4-126)
Eastern Cooperative Oncology Group performance status, n (%)			
≤2	9 (12)	5 (7)	14 (9)
3	27 (36)	28 (38)	55 (37)
4	17 (23)	20 (27)	37 (25)
Unknown	22 (29)	21 (28)	43 (29)
Primary disease, n (%)			
Acute myelogenous leukemia	18 (24)	29 (39)	47 (32)
Acute lymphoblastic leukemia	11 (15)	4 (5)	15 (10)
Chronic myelogenous leukemia	6 (8)	4 (5)	10 (7)
Other leukemia	1 (1)	0	1 (1)
Myelodysplastic syndrome	10 (13)	4 (5)	14 (9)
Non-Hodgkin lymphoma	10 (13)	9 (12)	19 (13)
Hodgkin lymphoma	3 (4)	6 (8)	9 (6)
Multiple myeloma	1 (1)	0	1 (1)
Aplastic anemia	1 (1)	1 (1)	2 (1)
Neuroblastoma	2 (3)	3 (4)	5 (3)
Immunodeficiency	3 (4)	0	3 (2)
Other	9 (12)	14 (19)	23 (15)
Previous treatment, n (%)			
Cyclophosphamide	61 (81)	58 (78)	119 (80)
Busulfan	32 (43)	31 (42)	63 (42)
Carmustine	0	4 (5)	4 (3)
Etoposide	7 (9)	5 (7)	12 (8)
Melphalan	8 (11)	15 (20)	23 (15)
Total body irradiation	33 (44)	35 (47)	68 (46)
Cyclosporine	38 (51)	35 (47)	73 (49)
Methotrexate	44 (59)	38 (51)	82 (55)
Sirolimus	13 (17)	14 (19)	27 (18)
Tacrolimus	30 (40)	24 (32)	54 (36)
Gemtuzumab ozogamicin	9 (12)	11 (15)	20 (13)
Number of transplantations, n (%)			
1	68 (91)	59 (80)	127 (85)
2	5 (7)	13 (18)	18 (12)
3+	2 (3)	2 (3)	4 (3)
Graft type, n (%)			
Autologous	8 (11)	12 (16)	20 (13)
Allogeneic	67 (89)	62 (84)	129 (87)
Days since transplantation, median (range)*	19 (6-48)	18 (0-60)	18 (0-60)

*At randomization.

who became dialysis- or ventilator-dependent during the trial had significantly lower CR and day +100 survival rates (Table 4).

Safety

Grade 3 or higher treatment-related AEs were reported for 2 patients in arm A and 3 patients in arm B ($P = .681$) (Table 5). No treatment-related deaths were reported. The incidence of grade 3 or higher expected AEs was 52% in arm A and 59% in arm B (Table 4), a statistically insignificant difference ($P = .411$). The most common grade 3–5 AEs overall were renal failure, hypotension, hypoxia, and other pulmonary events (Table 5); whereas all were less frequent in arm A events compared with arm B, with the difference being statistically significant only for hypoxia. Defibrotide-related AEs leading to discontinuation of treatment occurred in only 4% of patients (arm A: n = 2 [3%; hypotension and diffuse alveolar hemorrhage]; arm B: n = 4 [5%; hypotension, gastrointestinal bleeding, pulmonary hemorrhage, and abdominal cramps]).

The proportion of patients with treatment-related AEs was similar in adult and pediatric patients (8% in both). Analysis of specific event categories by patient age and treatment arm revealed that in pediatric patients, the incidence of bleeding events (68% vs 52%; $P = .377$) and hypotension (52% vs 26%; $P = .083$) was higher in arm B compared with arm A. The percentages of patients with all attributable AEs (likely or definitely related to defibrotide; 68% vs 39%; $P = .081$) and expected AEs (100% vs 74%; $P = .008$) were also higher in pediatric patients in arm B compared with those in arm A. Younger age

Table 2. Characteristics of VOD at Baseline (Treated Population) among All Patients and by Treatment Arm

Characteristic	Arm A (n = 75)	Arm B (n = 74)	All Patients (n = 149)
Days since total bilirubin ≥2 mg/dL, median (range)*	6 (−10 to 40)	4.5 (1-30)	5 (−10 to 40)
Total bilirubin, mg/dL, median (range)	6.4 (0.5-52.5)	6.3 (0.9-30.1)	6.4 (0.5-52.5)
Days since VOD diagnosis, median (range)*	2 (1-30)	2 (1-26)	2 (1-30)
Signs and symptoms, n (%)			
Jaundice	70 (93)	73 (99)	143 (96)
Ascites	54 (72)	55 (74)	109 (73)
>5% weight gain	60 (80)	55 (74)	115 (77)
RUQ pain	53 (71)	47 (64)	100 (67)
Hepatomegaly	56 (75)	49 (66)	105 (70)
Underwent liver biopsy, n (%)	16 (21)	10 (14)	26 (17)
WHVPG on liver biopsy, mm Hg, median (range)	23 (10-43)	18.5 (11-27)	23 (10-43)
SGOT/AST at hospital admission, U/L, median (range)	28 (8–233)	24 (9–836)	26 (8-836)
AST elevation at hospital admission, n (%)	17 (23)	14 (19)	31 (21)
SGPT/ALT at hospital admission, U/L, median (range)	26 (6-432)	28 (5-383)	27 (5-432)
Creatinine, mg/dL, median (range)	1.7 (0.2-5.0)	1.5 (0.2-5.5)	1.6 (0.2-5.5)
Abnormal creatinine, n (%)	53 (71)	48 (65)	101 (68)
Abnormal portal flow, n (%)	38 (51)	29 (39)	67 (45)
Requiring oxygen, n (%)	41 (55)	51 (69)	92 (62)
Ventilator-dependent, n (%)	6 (8)	4 (5)	10 (7)
Receiving dialysis, n (%)	3 (4)	4 (5)	7 (5)
Encephalopathy, n (%)	18 (24)	11 (15)	29 (19)
Organ systems compromised, n (%)			
1	1 (1)	2 (3)	3 (2)
2	37 (49)	39 (53)	76 (51)
3	34 (45)	30 (41)	64 (43)
4	3 (4)	3 (4)	6 (4)

AST indicates aspartate aminotransferase; RUQ, right upper quadrant; SGOT/AST, aspartate aminotransferase; SGPT/ALT, alanine aminotransferase; WHVPG, wedged transhepatic venous pressure gradient.

*At randomization. Negative values represent patients who did not have total bilirubin ≥2 mg/dL until after randomization (VOD diagnosed by liver biopsy rather than based on clinical criteria).

also was associated with higher rates of grade 3–5 bleeding events ($P = .015$) and grade 3–5 expected AEs ($P = .004$).

Pathology and Radiology

Fifty-two patients (47 of whom were evaluable for response) had liver specimens evaluable for pathology assessment. It should be noted that many of the specimens came from autopsies (22/52; 42%) or from patients who underwent biopsies because their conditions were not improving. The majority of patients sampled were confirmed to have VOD alone (63%) or VOD with another histological diagnosis (10%) (Table 6). Three other patients with small histological samples had features suggestive of VOD. One patient with marked hepatic GVHD had rare focal venular changes considered pathophysiologically insignificant. None of the 4 patients with the alternative diagnosis of GVHD without any other confounding features achieved CR, and none was alive at day

Table 3. Summary of Response and Day +100 Post-HSCT Survival among All Patients and by Treatment Arm

Characteristic	Arm A	Arm B	Total	*P* (A vs B)
CR, n/N (%) (evaluable population)				
Overall*	35/72 (49)	30/69 (43)	65/141 (46)	.613
Adult	19/49 (39)	19/45 (42)	38/94 (40)	.734
Pediatric	16/23 (70)	11/24 (46)	27/47 (57)	.100
Previous sirolimus/tacrolimus	12/28 (43)	9/22 (41)	21/50 (42)	.890
No previous sirolimus/tacrolimus	23/44 (52)	21/47 (45)	44/91 (48)	.469
Previous gemtuzumab ozogamicin	5/8 (63)	6/11 (55)	11/19 (58)	.729
No previous gemtuzumab ozogamicin	30/64 (47)	24/58 (41)	54/122 (44)	.542
Day +100 survival, n/N (%) (treated population)				
Overall	33/75 (44)	29/74 (39)	62/149 (42)	.619
Adult	17/52 (33)	20/49 (41)	37/101 (37)	.397
Pediatric	16/23 (70)	9/25 (36)	25/48 (52)	**.020**
Previous sirolimus/tacrolimus	9/31 (29)	9/24 (38)	18/55 (33)	.507
No previous sirolimus/tacrolimus	24/44 (55)	20/50 (40)	44/94 (47)	.158
Previous gemtuzumab ozogamicin	5/9 (56)	5/11 (46)	10/20 (50)	.653
No previous gemtuzumab ozogamicin	28/66 (42)	24/63 (38)	52/129 (40)	.616

HSCT indicates hematopoietic stem cell transplantation; CR, complete response.

*Of the 35 patients in arm A who achieved CR, 1 did so by day 7, 6 by day 14, 5 by day 21, 9 by day 28, and 14 after day 29. In arm B, the corresponding numbers were 2, 5, 0, 13, and 10 patients.

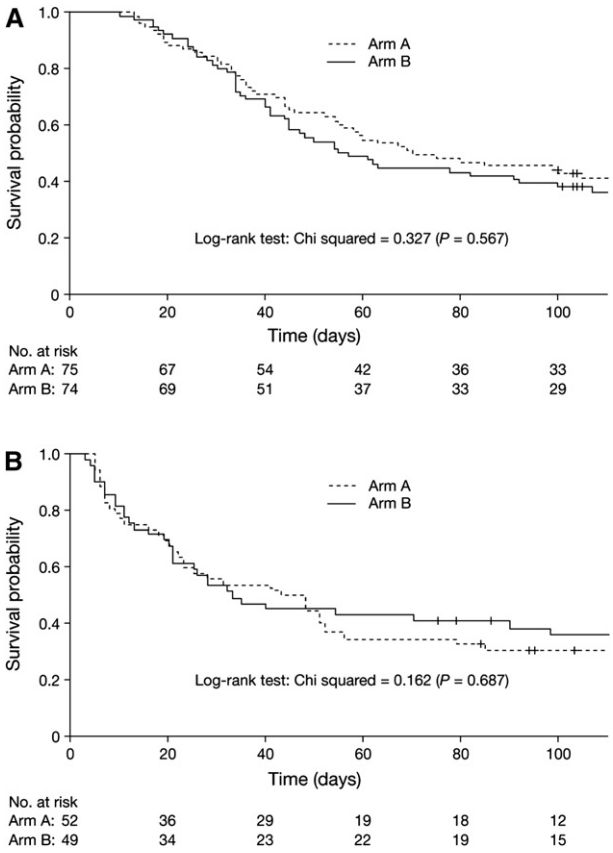

Figure 2. Survival of patients with severe hepatic VOD by treatment arm post-HSCT (A) and after randomization (B) (treated population). Kaplan-Meier curves show survival distributions for patients in treatment arms A and B.

+100. Overall, of the patients with confirmed VOD, 10 of 31 (32%) achieved CR, and 9 of 33 (27%) were alive at day +100. These percentages are lower than those for the overall population, possibly because sampling was biased toward patients with poorer clinical status and survival.

The distribution of baseline radiologic features was well balanced between the 2 treatment arms. At baseline, 99 of the 130 patients evaluated (76%) had ascites (75% in arm A and 78% in arm B), including 30 (23%) graded as trace/small (22% in arm A and 24% in arm B) and 69 (53%) graded as moderate/large (52% in arm A and 54% in arm B). By day 14, 70 of 97 patients (72%) had ascites, with 23 (24%) graded as trace/small and 47 (49%) graded as moderate/large. Absence of ascites at day 14 was seen in 18 of 51 patients (35%) who achieved CR and in 17 of 47 patients (36%) who were alive at day +100. Multivariate stepwise selection analysis revealed an association between the absence of ascites at day 14 and better response ($P = .070$). Hepatic venous flow was abnormal (defined as a loss of the normal triphasic pulsatility pattern) in 36 of 105 patients (34%) at baseline (37% in arm A and 32% in arm B). At day 14 of treatment, 17 of 81 patients (21%) had abnormal flow, including 13 of 46 (28%) who achieved CR and 14 of 43 (33%) who were alive at day +100. Abnormal hepatic venous flow at baseline was not associated with better response or survival, but abnormal hepatic venous flow at day 14 emerged as a predictor of better outcome by multivariate stepwise selection analysis, associated with both better response ($P = .047$) and day +100 survival ($P = .007$). At baseline, portal venous flow was abnormal in 92 of 129 patients (71%). At day 14, 51 of 98 patients (52%) had abnormal portal flow, including 24 of 53 (45%) who achieved CR and 21 of 49 (43%) who were alive at day +100.

Changes in PAI-1 and Total Bilirubin Levels

Changes in PAI-1 and total bilirubin levels with defibrotide treatment were noted (Figure 4A and B). There were no significant differences in mean PAI-1 level by treatment arm, between patients with or without CR, or between patients alive or dead at day +100 (Figure 4A); however, mean levels at days 7 and 14 were lower (although not statistically significantly so) compared with baseline in patients with CR and patients alive at day +100, with a similar pattern observed in both treatment arms.

Pharmacokinetics

Samples for PK analysis were obtained from 11 patients (8 adult and 3 pediatric patients; 6 patients in arm A and 5 in arm B). Throughout the PK sampling period (days 1, 2, and 7), defibrotide concentration in plasma samples remained constant at approximately 200 μg/mL, consistent with values obtained for therapeutic dosing in animal studies. No marked difference in defibrotide concentration was seen between treatment arms or between dialysis (n = 4) and nondialysis patients (n = 7).

DISCUSSION

In this prospective randomized phase II trial, the largest of its kind to date in patients with severe VOD after HSCT, defibrotide was administered for up to 82 days at 25 mg/kg/day and for up to 65 days at 40 mg/kg/day, and was well tolerated overall. There were no deaths (including hemorrhage) considered related to defibrotide. The most common reason for discontinuing defibrotide treatment was resolution of VOD (32% of patients overall); only 4% of patients discontinued treatment because of defibrotide-related toxicity. The 25-mg/kg/day dosing regimen had a more favorable safety profile than the 40-mg/kg/day regimen, most notably in pediatric patients, with a lower incidence of AEs.

Defibrotide was effective; overall, 46% of the evaluable patients achieved CR, and 42% of the treated patients were alive at day +100 post-HSCT. There

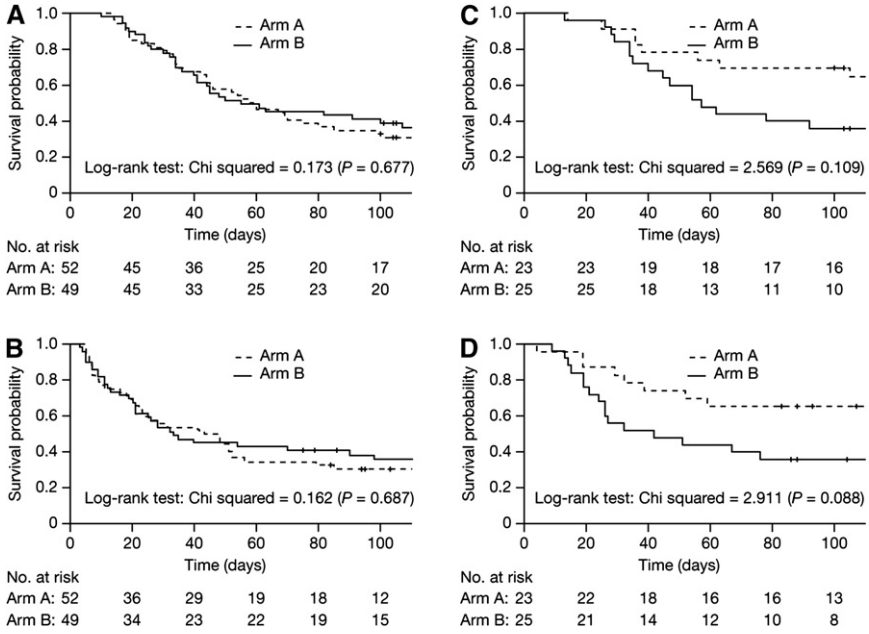

Figure 3. Survival of patients with severe hepatic VOD by treatment arm for adult patients post-HSCT (A) and after randomization (B) and for pediatric patients post-HSCT (C) and after randomization (D) (treated population). Kaplan-Meier curves show survival distributions for patients aged ≥18 years and aged <18 years.

were no significant differences between the 2 treatment arms in CR or day +100 survival rates, or in survival distributions after HSCT or after randomization. Given the very high mortality typically associated with severe VOD [5,6], as well as the presence of MOF in all but 3 patients at initiation of treatment, these results are very promising and are consistent with previous findings from smaller clinical trials of defibrotide in the treatment of severe VOD/MOF [26-30].

Little has been published on the factors predicting resolution or response to treatment of severe VOD. Greater weight gain and higher bilirubin levels have been associated with the development of severe VOD and poorer survival [31], whereas in pediatric patients with VOD, predictors of mortality include an HSCT donor other than autologous or matched sibling, presence of hepatic and cutaneous GVHD, maximal weight gain >9%, presence of pleural effusion, intensive care, and a higher and/or more rapid increase in bilirubin level [33]. In this trial, CR and day +100 post-HSCT survival rates were higher in pediatric patients than adult patients, particularly in arm A. An association between younger age and better outcome also has been reported in a previous study of defibrotide [28], as well as in studies with other treatments [8,34,35]. Although previous gemtuzumab ozogamicin therapy is known to be a risk factor for development of severe VOD [36], less is known about the subsequent response to treatment. The results of the present study suggest that defibrotide may provide an effective treatment option for patients who develop severe VOD after exposure to this agent. Sirolimus combined with tacrolimus treatment was associated with decreased day +100 survival, however. This may be in part because these agents were continued during treatment, a practice that has since changed, with sirolimus now recognized as an important risk factor for VOD [37,38]. This increased risk of VOD may be associated with sirolimus' potent effects on inhibiting endothelial cell function, accelerating

Table 4. Summary of Response and Day +100 Post-HSCT Survival by Dialysis or Ventilator Dependence at Baseline and on Study, among All Patients and by Treatment Arm

Characteristic	Arm A	Arm B	All Patients	*P* vs Overall Population
CR, n/N (%) (evaluable population)				
Dialysis dependence at baseline	0/3 (0)	2/4 (50)	2/7 (29)	.352
Dialysis dependence on study	7/28 (25)	7/30 (23)	14/58 (24)	.001
Ventilator dependence at baseline	1/6 (17)	1/4 (25)	2/10 (20)	.098
Ventilator dependence on study	4/26 (15)	6/27 (22)	10/53 (19)	<.001
Day +100 survival, n/N (%) (treated population)				
Dialysis dependence at baseline	1/3 (33)	2/4 (50)	3/7 (43)	.946
Dialysis dependence on study	6/30 (20)	8/32 (25)	14/62 (23)	.002
Ventilator dependence at baseline	1/6 (17)	1/4 (25)	2/10 (20)	.166
Ventilator dependence on study	5/27 (19)	7/29 (24)	12/56 (21)	.002

HSCT indicates hematopoietic stem cell transplantation; CR, complete response.

Table 5. Summary of Treatment-Emergent AEs in Patients with Severe Hepatic VOD after HSCT (Treated Population), Overall and by Treatment Arm

AE	Arm A (n = 75)	Arm B (n = 74)	All Patients (n = 149)	*P* (A vs B)
Any AE, n (%)	71 (95)	73 (99)	144 (97)	0.367
Grade 3-4	64 (85)	68 (92)	132 (89)	0.303
Grade 5	12 (16)	14 (19)	26 (17)	0.671
Most common grade 3-5 AEs, n (%)				
Renal failure	19 (25)	27 (37)	46 (31)	0.159
Hypotension	20 (27)	23 (31)	43 (29)	0.591
Hypoxia	13 (17)	25 (34)	38 (26)	0.025
Pulmonary–other	16 (21)	17 (23)	33 (22)	0.846
Treatment-related AEs, n (%)	5 (7)	7 (10)	12 (8)	0.563
Grade 3-4	2 (3)	3 (4)	5 (3)	0.681
Grade 5	0	0	0	–
Treatment-related bleeding events, n (%)	0	1 (1)	1 (1)	0.497
Grade 3-4	0	1 (1)	1 (1)	0.497
Grade 5	0	0	0	–
Expected AEs, n (%)	56 (75)	64 (87)	120 (81)	0.097
Grade 3-4	38 (51)	43 (58)	81 (54)	0.412
Grade 5	1 (1)	1 (1)	2 (1)	1.000

AEs indicate adverse events; VOD, veno-occlusive disease; HSCT, hematopoietic stem cell transplantation.

senescence of hepatic endothelial cells in response to injury, preventing healing of hepatic sinusoids after endothelial injury, and/or increasing the risk of thrombotic microangiopathy [37,39]. As might be expected, patients in our trial with advanced MOF, as reflected by baseline or acquired ventilator and/or dialysis dependence, had inferior CR and day +100 post-HSCT survival rates, strongly suggesting that earlier intervention may be beneficial.

Of note, based on radiologic examination, abnormal (ie, blunted) hepatic venous flow at day 14 of defibrotide treatment, but not at baseline, was associated with improved outcome in our trial, whereas abnormal portal flow (ie, increased pulsatility or flow reversal) was not. This may reflect the dominance of acute hepatic edema as opposed to fibrosis in cases of reversible VOD. In contrast, portal flow changes can have multiple causes and are more remote (upstream) from the primary site of damage, the hepatic sinusoid, and thus may imply the presence of more extensive (or irreversible) sinusoidal dysfunction. Future analyses of radiologic findings, including assessment of hepatic artery resistive indices and correlation of radiologic features over time with clinical response, are planned.

Possible early predictive biomarkers for VOD development have been identified, including elevated serum PAI-1, elevated N-terminal propeptide of type III procollagen, elevated t-PA, and low protein C level [40,41]. In our trial, PAI-1 levels at days 7 and 14 were decreased baseline levels prior to the start of treatment in patients who subsequently responded and were alive at day +100 post-HSCT. In addition, early stabilization of or decreased bilirubin concentration was associated with better overall outcome. This finding appears to be consistent with predictive biomarkers for VOD development and prognosis, as well as with known effects of defibrotide. These findings suggest that early changes in both bilirubin and PAI-1 may help predict response, and also might be useful in early identification of individuals in whom additional, alternative, or experimental therapies should be considered during defibrotide treatment. Additional exploratory studies of predictive biomarkers in this trial have been carried out, and future studies to incorporate assessment of angiogenic cytokines, including vascular endothelial growth factor and fibroblast growth factor, along with analyses of additional endothelial stress markers, are planned [24].

Given the paucity of viable treatment options for VOD, as well as the limited nature of supportive care in this syndrome, defibrotide represents the most promising therapy for VOD to date, with a recent large European compassionate-use program also reporting positive results [42]. In addition to defibrotide's potential for treating established VOD as a single agent, future research directions include the use of defibrotide in combination with other agents demonstrating possible efficacy, such as human protein C concentrate, N-acetylcysteine, and methylprednisolone [34,43,44]. Importantly, initial studies of defibrotide in VOD prophylaxis have suggested efficacy, with

Table 6. Summary of Central Pathology Review Findings

Pathology Finding	All Patients with Pathology Assessment (Evaluable for Day +100 Post-HSCT Survival) (n = 52)*	Patients with Pathology Assessment Who Were Evaluable for Response (n = 47)*
VOD, n (%)	33 (63)	31 (66)
VOD plus another histologic diagnosis, n (%)	5 (10)	5 (11)
Findings consistent with, but not diagnostic of, VOD, n (%)	3 (6)	3 (6)
Alternative diagnosis, n (%)†	10 (19)	8 (17)
GVHD, n (%)	4 (8)	4 (9)
Insignificant for diagnosis, n (%)	1 (2)	0 (0)

HSCT indicates hematopoietic stem cell transplantation; VOD, veno-occlusive disease; GVHD, graft-versus-host disease.

*One patient had no VOD on liver biopsy and was excluded before initiation of defibrotide. One patient had no VOD on liver biopsy performed after initiation of defibrotide; this patient was removed from the trial.

†In addition to the 4 patients with possible, probable, or definite GVHD, alternative diagnoses were cirrhosis in 2 patients and viral hepatitis, shock, abscesses, and GVHD or cholangitis lenta each in 1 patient.

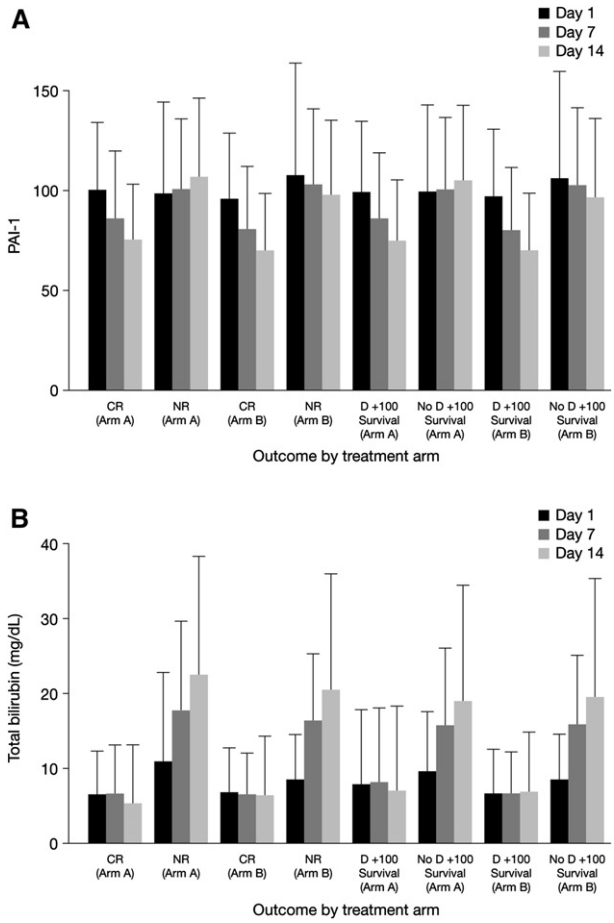

Figure 4. Mean (standard deviation) PAI-1 (A) and total bilirubin (B) by treatment arm and outcome in patients with severe hepatic VOD treated with i.v. defibrotide. Bar charts illustrate PAI-1 levels (A) and total bilirubin levels (B) by CR/nonresponse, day +100 post-HSCT survival/nonsurvival, and treatment arm. D, day; NR, nonresponder.

significant reductions in the incidence of VOD [45-48]; a prospective randomized, phase II/III trial has explored prophylactic defibrotide in children undergoing HSCT with encouraging preliminary results [49,50].

In conclusion, the results of this dose-finding trial demonstrate that defibrotide at a dose of either 25 or 40 mg/kg/day appears to be effective in treating severe VOD after HSCT, with low treatment-related toxicity. As specified a priori, the 25-mg/kg/day dose has been selected for use in Phase III trials of defibrotide in VOD, based on its more favorable safety profile. A large Phase III trial of defibrotide at this dose using a historical-control methodology has recently been completed in patients with severe VOD and MOF, with final results anticipated soon [51,52].

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research and collected data, analyzed data, and wrote and approved the manuscript.

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SUPPLEMENTARY DATA

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbmt.2010.02.009

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